REVIEW

Somatic mutations of the epidermal growth factor receptor and non-small-cell lung cancer

Xiaozhu Zhang, Alex Chang

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Frequent overexpression of epidermal growth factor receptor (EGFR) in non-small-cell lung cancer (NSCLC) makes EGFR a new therapeutic target. Two specific EGFR tyrosine kinase inhibitors, gefitinib (ZD1839, Iressa) and erlotinib (OSI-774, Tarceva), have been developed and approved by the US Food and Drug Administration for second-line and third-line treatment of advanced NSCLC. Clinical trials have shown considerable variability in the response rate between different patients with NSCLC, which led to the discovery of somatic *EGFR*-activating mutations. This brief review summarises the discovery and functional consequences of the mutations, their clinicopathological features and significant implications in the treatment and prognosis of NSCLC.

See end of article for authors' affiliations

Correspondence to: Dr X Zhang, Division of Biomedical Sciences, Johns Hopkins Singapore, 31 Biopolis Way, #02-01, the Nanos, Singapore 138669, Singapore; xiaozhu@imc.jhmi.edu

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Lung cancer is one of the most common human cancers and the leading cause of death due to cancer worldwide.[1] It claims more lives than colorectal, breast and prostate cancers combined. Fewer than 15% of patients can be cured and have a >5 year survival rate, as the disease is often in an advanced stage at the time of diagnosis and chemotherapy/radiotherapy cannot cure the advanced disease.[2] Lung cancer is generally classified into two basic types, small-cell lung cancer and non-small-cell lung cancer (NSCLC). NSCLC accounts for approximately 85% of cases and can be further divided into squamous-cell carcinoma, adenocarcinoma and large-cell carcinoma.[3] In recent years, adenocarcinoma has replaced squamous-cell carcinoma as the most common histological subtype of NSCLC in the US and many other parts of the world.[4 5]

The pathogenesis of lung cancer is yet to be fully understood. Multiple environmental factors are implicated in lung carcinogenesis, such as outdoor and indoor pollution, diet and bacterial and viral infections.[6] Cigarette smoke has been widely accepted as the major cause of lung cancer and a linear dose-response relationship has been established between risk of lung cancer and the amount of cigarettes consumed.[7–9] The occurrence of lung cancer is also closely associated with the accumulation of multiple genetic and/or epigenetic changes.[10–12] Better understanding of molecular mechanisms underlying the pathogenesis of lung cancer would provide pivotal guidance for the treatment and prevention of cancer.

EGFRs AND LUNG CANCER

Human tumours often express high levels of epidermal growth factor (EGF)-related receptors, which include EGFR/HER1, c-erbB2/HER2, c-erbB3/HER3 and c-erbB4/HER4 (table 1).[16–20] All these receptors share a common extracellular ligand-binding domain, a hydrophobic transmembrane domain and a multifunctional intracellular domain that has an ATP-binding site and tyrosine kinase activity (HER3, however, does not have tyrosine kinase activity). Receptor activation is generally initiated by the binding of EGF-related ligands and receptor homodimerisation or heterodimerisation, which activate the intrinsic tyrosine kinase activity of the receptors and lead to autophosphorylation of the tyrosine residues. These residues further activate downstream signalling cascades, such as the Ras-Raf-MAP-kinase, PI3K-Akt and STAT pathways, which have strong regulatory effects on cell proliferation, differentiation, survival and migration.[21–23]

EGFR is generally expressed at a low level in a wide variety of normal tissues. Excessive expression or activation of EGFR is able to induce malignant transformation.[24] Overexpression of EGFR has been observed in 40–80% of NSCLCs,[20 25] and it is often associated with aggressive clinical behaviours, such as advanced stage, increased metastatic rate, higher tumour proliferation rate and poor prognosis.[26–29] Other than overexpression, a mutant form of EGFR (EGFRvIII) with constitutive tyrosine kinase activity has been identified in NSCLC[30–32] and is implicated in lung tumorigenesis.[33 34]

Given that EGFR is often overexpressed in NSCLC and given its potential role in lung carcinogenesis, EGFR has been considered a rational target molecule for the treatment of NSCLC. Specific EGFR tyrosine kinase inhibitors (EGFR-TKIs), gefitinib and erlotinib, have been approved by the US Food and Drug Administration as monotherapy treatment for advanced or metastatic NSCLC.[35 36] Both agents adversely compete with ATP for the critical ATP-binding site located in the intracellular domain and inhibit receptor phosphorylation.

GEFITINIB AND *EGFR* MUTATIONS

The effectiveness of gefitinib has been evaluated by two randomised and double-blinded Phase II clinical trials in patients with advanced NSCLC.[37 38] The results suggest that gefitinib is a well-tolerated oral EGFR-TKI. It has meaningful antitumour activity and brings about considerable

Abbreviations: EGF(R), epidermal growth factor (receptor); NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; TKI, tyrosine kinase inhibitor

Table 1 The family of human epidermal growth factor (EGF)-related receptors

	Gene location	Molecular weight	Protein distribution
c-erb B-1/EGFR	7p12.3–p12.1	170 kDa	A large variety of cell type or tissues except haemopoietic cells[13]
c-erb B-2/HER-2	17q21.1	185 kDa	A number of human secretory epithelial tissues[14]
c-erb B-3/HER-3	12q13	160 kDa	Several human tumour cell lines[15]
c-erb B-4/HER-4	2q33.3–q34	180 kDa	Lining epithelia of the gastrointestinal, urinary, reproductive, breast cancer cell lines, and normal skeletal muscle, heart, pituitary, brain and cerebellum[16]

improvement in cancer-related symptoms in certain subgroups of patients (approximately 10–19%). Females, non-smokers, Japanese people and patients with lung adenocarcinoma generally have a higher response rate than males, smokers, people of European origin and patients with other histological types of NSCLC.[35 37 38] To determine whether somatic mutations in the *EGFR* gene play a causal role in response to TKI treatment, two research groups have systematically sequenced all 28 exons of *EGFR* and identified several important activating mutations that show striking correlation with gefitinib response.[39 40] This discovery has been claimed as the most significant molecular event in lung cancer.[41] It has greatly stimulated research in this area worldwide, and a number of other novel mutations have been identified (table 2).

Genotyping methods

Two important factors affect the detection of somatic *EGFR* mutations in clinical cancer samples. The first is the availability of the tumour genome. There is no doubt that frozen surgical tumour samples[45] and tumour paraffin blocks[47] are the best samples for mutation analysis, as they are directly resected from corresponding tumours and can provide sufficient tumour nucleic acids for genotyping. However, a large proportion of patients with NSCLC are not eligible for surgery on diagnosis. Therefore, non-surgical specimens, such as diagnostic biopsy and effusion drainage, are probably as important as surgical specimens in these patients with advanced cancer. Pleural effusion[56] and needle biopsy/aspiration[49] have been successfully managed for mutation screening. Asano *et al*[57] even showed the feasibility of detecting *EGFR* mutations with the use of soluble DNA extracted from pleural fluid.

The second factor affecting mutation detection is the purity of the tumour genome. Usually clinical cancer samples contain a large proportion of normal cells, which make up a strong background of wild-type alleles and seriously dilute the signal from biologically important somatic mutations. Therefore, the sensitivity of genotyping methods is of great importance for the detection of mutations.

Among a number of reported methods, PCR-based direct sequencing is the most commonly used.[39 40 43 44 47] With the help of cloning technology, even samples presenting difficulty in direct sequencing can be sequenced using primers located on vectors. Moreover, tumour RNA can be used for genotype determination, as all the reported *EGFR*-activating mutations are exonic.[42 55] However, RNA is usually more difficult to handle than genomic DNA, because of its rapid degradation and limited quantity.

Single-strand conformation polymorphism (SSCP) assay is another important method used for *EGFR* mutation screening. SSCP has been considered to be more sensitive than direct sequencing in mutation analysis.[58 59] The two large studies performed by Marchetti *et al*[46] and Sonobe *et al*[48] have reported that SSCP assays not only confirmed all the *EGFR* mutations detected by direct sequencing but also identified additional mutations that were missed in sequencing analysis. Therefore, SSCP assay could be a reliable method for large-scale diagnostic screening for *EGFR* mutations in clinical samples.

For detection of known *EGFR* mutations, a number of methods have been developed, including restriction fragment length polymorphism and length analysis of fluorophore-labelled PCR products,[60] peptide nucleic acid–locked nucleic acid PCR clamp,[61] mutant-allele-specific amplification[62] and mutant-enriched PCR.[57] The restriction fragment length polymorphism–capillary electrophoresis method can not only detect the mutations but also estimate gene amplification based on the relative height of the mutation peak to the germline peak. The peptide nucleic acid–locked nucleic acid PCR clamp, mutant-allele-specific amplification and mutant-enriched PCR have high sensitivity. They are able to distinguish even one *EGFR* mutant tumour cell in the presence of up to 1000–2000 normal cells.[57 61 62]

The pattern and functional consequence of *EGFR* mutations

Three common types of *EGFR* mutation—in-frame deletion, insertion and missense mutation—have been identified. Most of the mutations are located in the tyrosine kinase-coding domain (exons 18–21). Amino acids 746–753 encoded by exon 19 and amino acid 858 encoded by exon 21 are the two mutation hotspots, comprising >80% of the mutations. All the identified mutations are of somatic origin, and not present in the germline genome.

EGFR mutations have been proposed as an early event in lung carcinogenesis. They are not correlated with the classification of tumour stage.[46] Well or moderately differentiated tumours have more *EGFR* mutations than poorly differentiated tumours.[48] Some of the mutations can even be detected in respiratory epithelia with normal histology.[63] The oncogenic characteristics of *EGFR* mutants have recently been proved by anchorage-independent growth and focus formation in transfected cells and tumour formation in immunocompromised mice.[64]

EGFR mutants (deletion in exon 19 and L858R) are capable of enhancing EGF-dependent receptor activation (Tyr^{1068}).[40] The downstream signalling pathways Akt and STAT are also selectively activated,[65 66] and these have an important anti-apoptotic function. When mutant EGFR expression is suppressed by specific small interfering RNA or when Akt and STAT pathways are blocked by specific inhibitors, rapid and massive apoptosis occurs. A similar event also happens when EGFR-TKIs are applied to mutant NSCLC cell lines.[51 65 66] All these suggest that excessive EGFR signalling plays a critical role in tumorigenesis in patients harbouring an *EGFR* mutation, and mutant EGFRs drive the growth of cancer cells and maintain their malignant phenotype by the selective activation of Akt and STAT signalling pathways.

EGFR gene amplification is present in approximately 30–40% of patients with NSCLC,[67 68] and it is associated with a susceptibility to gefitinib.[68–70] The association between *EGFR* mutations (deletion in exon 19 and L858R in exon 21) and an increased copy number of the *EGFR* gene has been shown in both preclinical[66 71] and clinical investigations,[50 70 71] which could partially throw light on gefitinib sensitivity in patients with *EGFR* gene amplification. Genetic instability has been

Table 2 Mutations identified in exons 18–21 of the *EGFR* gene (RefSeq NM_005228)*

Sequence change	Amino acid change	Reference
Exon 18		
2126A→T	E709V	42, 43
2126A→C	E709A	44, 45
2126A→G	E709G	44, 45
2125G→A	E709K	45
2155G→A	I715S	43
2155 G→T	G719C	40, 42–47
2155 G→A	G719S	39, 43–45
2156 G→C	G719A	42–44, 47, 48
2159 C→T	S720F	43, 48
Exon 19		
2225T→C	V742A	49
Del (2236–2244)+2245G→C+2248G→C	E746-R748 del with E749Q, A750P	47
Del (2235–2249)/del (2236–2250)	E746-A750 del	39, 40, 42–50
Del (2235–2236)+ Del (2242–2248)+2241A→C	E746-A750 del with I, P ins	48
Del (2237–2251)+2252C→T	E746-A750 del with V ins	43
Del (2237–2251)	E746-T751 del with A ins	44–46, 48, 50
Del (2235–2236)+ Del (2239–2252)/ Del (2235–2252)+ 2254T→A+2255C→T	E746-T751 del with I ins	43, 48
Del (2235–2236)+ Del (2242–2248)+2241A→C	E746-T751del with I, P ins	48
2237–2238 AA→TC+ Del (2239–2253)	E746-T751 del with V ins	43, 49
Del (2484–2501)	E746-S752 del with D ins	44
Del (2237–2254)+2255C→T	E746-S752 del with V ins	45, 48, 50
Del (2239–2247)	L747-E749	46
Del (2239–2247)+2248G→C	L747-E749 del with P ins	39, 42, 43, 45
Del (2240–2248)+2239T→C	L747-T750 del with P ins	43, 48
Del (2238–2252)/ Del (2239–2253)/ Del (2240–2254)	L747-T751 del	43, 44, 48, 49
Del (2240–2251)	L747-T751 del with S ins	40, 46, 48
Del (2239–2256)	L747-S752 del	44, 48
Del (2239–2256)+2258 C→A	L747-S752 del with Q ins	43
Del (2238–2255)+2237A→T	L747-S752 del, E746V	39
Del (2240–2257)	L747-P753 del with S ins	39, 40, 42–46, 48–50
Del (2240–2257)	L747S, R748-P753 del	47
Del (2254–2277)	S752-I759 del	39, 47
2273A→G	E758G	49
Exon 20		
2308 ins GCCATA	M766-A767 with AI ins	47
2308 ins CCAGCGTGG+ 2310C→T silent	A767-S768 with SVA ins	47
2303G→T	S768I	43–45, 47
Dup (2549–2557)	S768-D770 dup	44
2308–2316 ins GCCAGCGTG	ASV770-772 ins	43
2320–2322 ins CAC	H774 ins	43
2317–2222 ins AACCCC+ 2223 C>T	NP773-774 ins, H775Y	43
2320–2325 ins CCCCAC	PH774-775 ins	43
2320–2328 ins AACCCCCAC	NPH774-776 ins	43
2326C→T	R776C	51
2308 ins (CCAGCGTGG)+ 2310C→T+ 2315C→G	ins779 ASV+P782R	48
2311 ins (GCGTGGACA)+ 2315C→G	ins780 SVD+P782R	48
2369 C→T	T790M	52–55
Exon 21		
2743T→G	L833V	44
2750A→T	H835L	44
2758C→G	L838V	44
2513T→C	L838P	49
2520C→T	A840A	49
2527G→A	V843I	49
2551G→A	V851I	49
2572C→A	L858M	46
2573T→G	L858R	39, 40, 42–50
2582T→A	L861Q	40, 42–45
2593G→A	E856L	49
2612C→T	A871V	49

*Only mutations reported with both nucleotide and amino acid changes are summarised. The beginning of the coding sequence is defined as +1.

proposed to be the cause of gene amplification and the facilitator of the incidence of *EGFR* mutations.[71]

FACTORS PREDISPOSING TO *EGFR* MUTATIONS IN NSCLC

The distribution of *EGFR* mutations in NSCLC has been intensively investigated (table 3). In general, *EGFR* mutations are more common in patients of oriental origin, in females and in patients with a history of never smoking or with adenocarcinoma.[73]

Ethnicity

EGFR mutations have significant ethnic variation. The mutation rate is approximately 5–13% among Caucasians,[43 46 47] but 30–40% among East Asians (table 3).[43 44 55] Although the factors that determine more *EGFR* mutations in Asians are still an enigma, different genetic backgrounds and living environments could provide informative clues. The international HapMap project (http://www.hapmap.org) has genotyped >300 single-nucleotide polymorphisms across the *EGFR* gene in Caucasian, Japanese, Chinese Han and Yoruba populations. Considerable interethnic variations in the prevalence of single-nucleotide polymorphisms have been observed between Japanese and Caucasian populations (fig 1). It remains unknown if the germline variations are correlated with the occurrence of *EGFR* somatic mutations. However, the association between germline and somatic variations in upper gastrointestinal familial adenomatous polyposis has been reported.[74]

Smoking status and sex

Cigarette smoke is the most significant risk factor for lung cancer. N-nitrosamines and polycyclic aromatic hydrocarbons, the two major classes of tobacco-related carcinogens, can produce as many as 100 mutations per cell genome by means of formation of DNA adducts.[75] Cigarette smoke, however, is not the mutagen of *EGFR,* as *EGFR* mutations are usually more frequent in patients who never smoke or have a low exposure to cigarette smoke (table 3). Risk factors associated with non-smoking lung cancer, such as pre-existing lung diseases, a family history of cancer, passive smoking, indoor cooking fumes and occupational exposures[76] could affect the occurrence of *EGFR* mutations. More studies are required to identify the causal mutagens and elucidate the potential mutagenic capability.

EGFR mutations are more frequent in female than in male patients, especially among Asians (table 3). This difference could be correlated with their distinct life styles and smoking habits. Generally, women tend to be non-smokers or light smokers, and they play a heavier role in housework, such as cooking and cleaning, whereas those men who smoke tend to be heavy smokers and are more often involved in social activities. If the occurrence of *EGFR* mutations is associated with potential indoor mutagens, there is no doubt that women would have a higher mutation rate than men. On the other hand, female endocrine factors could also play a role in *EGFR* mutations.

Histology of NSCLC

EGFR mutations are more frequent in lung adenocarcinoma than in other histological types of NSCLC (table 3). As the predominant subtype of NSCLC, adenocarcinoma usually originates from a peripheral airway compartment, either surface epithelium or bronchial mucosal glands.[77] It is likely that the specific cellular milieu in these cell types is more susceptible to the influence of mutagens that induce *EGFR* mutations compared with other cell types.

CLINICAL SIGNIFICANCE OF *EGFR* MUTATIONS

EGFR mutations have been considered the best predictive marker for response to treatment with EGFR-TKIs in NSCLC. A number of retrospective studies have indicated that patients with the mutations have higher objective response rates to gefitinib than patients with the wild type (table 4). The first prospective clinical trial has recently been conducted among 75 patients with chemotherapy-naive advanced NSCLC in Japan. Among 25 patients with *EGFR* mutations, 16 received gefitinib monotherapy and nine received standard chemotherapy. In agreement with the retrospective studies, the group receiving gefitinib had a 75% response rate to gefitinib.[78] Another recent prospective Phase II trial of gefitinib has shown similar results.[79]

Sensitivity to gefitinib or erlotinib is also associated with the type of mutation and the existence of additional mutations.

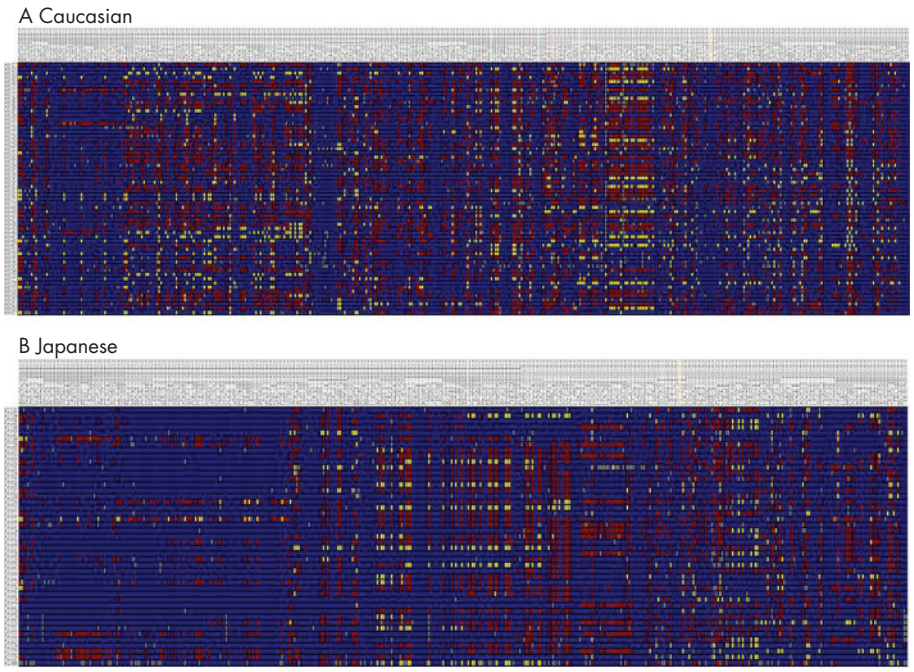

Figure 1 Genotype distribution of germline *EGFR* polymorphisms in Caucasian and Japanese populations. The genotype datasets are downloaded from the HapMap project and displayed in Visual Genotype (VG) format (http://pga.gs.washington.edu). The columns are the polymorphic sites. The rows are the arrays of samples. Blue represents a homozygote for the common allele, red represents a heterozygote (both common and rare allele) and yellow represents a homozygote for the rare allele.

Table 3 Overview of 10 large studies on *EGFR* mutations in non-small-cell lung cancer (NSCLC)

	Ethnicity	Subjects (n)	Mutation rate (%)	Deletions in exon 19 (%)	L858R (%)	Mutation rate (%) Non-smokers vs smokers	Female vs male	Adenocarcinoma vs other NSCLC
Marchetti[46]	White	860	5	46	46	20 vs 2	19 vs 2	10 vs 0
Eberhard[47]	White	228	13	62	24	25 vs 12	13 vs 13	15 vs 11
Cappuzzuo[67]	White	89	17	53	47	46 vs 12	26 vs 12	20 vs 10
Shigematsu[43]	White	158	8	46	39	29 vs 3	20 vs 1	16 vs 1
	East Asian	361	30			56 vs 14	55 vs 19	48 vs 3
Kosaka[55]	Japanese	277	40	47	44	66 vs 22	59 vs 26	49 vs 2
Sonobe[48]	Japanese	154	39	57	37	79 vs 16	70 vs 19	55 vs 0
Tokumo[72]	Japanese	120	32	50	47	69 vs 15	57 vs 20	45 vs 3
Huang[44]	Taiwanese	101	39	33	51	47 vs 18	44 vs 32	55 vs 3
Sugio[62]	Japanese	469	29	45	55	54 vs 19	46 vs 21	42 vs 1
Yokoyama[45]	Japanese	349	29	40	39	54 vs 14	54 vs 15	42 vs 1

Patients with deletion mutation in exon 19 have higher response rates than those with L858R in exon 21.[42 80] An additional mutation in exon 22 (K884E) is able to make tumour cells sensitive to gefitinib but resistant to erlotinib.[81] Further laboratory and clinical studies are required to elucidate the underlying mechanisms.

Furthermore, *EGFR* mutations have been considered a favourable prognostic indicator in NSCLC. Patients with the mutations have improved time-to-progression and longer overall survival than those with the wild type (table 4).[42 49 50 67 70] However, two Japanese studies have found conflicting results, namely that there is no significant difference in survival between the groups with and without the mutations.[43 62] This discrepancy could reflect the fact that other prognostic factors besides *EGFR* mutations also play an important role in survival among patients with NSCLC.

SECONDARY *EGFR* MUTATIONS AND ACQUIRED RESISTANCE TO GEFITINIB

Gefitinib has brought significant treatment response among patients with NSCLC with *EGFR* mutations. However, the mean duration of response is generally 6–8 months.[37 38] Most of these patients eventually relapse and are resistant to further treatment with EGFR-TKIs. The acquired resistance is closely associated with the development of a secondary mutation in exon 20, that is, a substitution of methionine for threonine at amino acid position 790 (T790M).[52 53] Pao *et al*[52] have reported that three of six patients with acquired resistance to TKIs harbour the T790M mutation in the progressing tumours. T790M is believed to abrogate the binding of TKIs to the ATP-kinase-binding pocket and lead to the continued activation of ErbB-3/PI3K/Akt signalling.[53 82] The scenario is analogous to the secondary mutations in *BCR-ABL* and *KIT* that confer acquired resistance to imatinib (Gleevec) in chronic myeloid leukaemia and gastrointestinal stromal tumour.[83 84] T790M has been considered to be present only in relapsed tumours and its appearance to be secondary to treatment of TKIs. Nevertheless, rare T790M mutation has been detected in untreated tumour[55] and even in germline DNA, and it is associated with family aggregation of NSCLC.[54] Therefore, T790M could play a role in lung tumorigenesis.

ASSOCIATION OF *EGFR* MUTATIONS WITH *K-ras* AND *P53* GENE MUTATIONS

K-ras is a key molecule in the signalling pathways, which regulate cellular proliferation and transformation. The *K-ras* mutation is one of the major genetic changes detected in lung cancer. The mutations are more common in adenocarcinomas, are closely correlated with cigarette smoke and are predominantly the guanine-to-thymine transversion at codon 12.[85 86] Cigarette smoke has been proposed as the causal mutagen. *P53* is another well-characterised gene in lung cancer. Most of the mutations are located in evolutionally conserved regions, and the mutation spectra are different between smokers and non-smokers. The relationship between the type of *p53* mutations and the histology of lung cancer is still unclear.[87 88]

The correlation between *EGFR* mutations and *K-ras* mutations has been intensively investigated. Generally, *EGFR* mutations are present only in lung adenocarcinomas which do not harbour *K-ras* mutations.[43 46–48 55 62] This observation might imply that there are different subsets of lung adenocarcinomas, which possess different mutation spectra and causal mutagens. By contrast, mutations in the *EGFR* gene and the *p53* gene happen independently,[48 55] but the status of gene–gene interaction and its influence on lung carcinogenesis and treatment response remains unknown.

Table 4 *EGFR* mutations, clinical tyrosine kinase inhibitor response and survival

	Mutation/EGFR-TKI responders (%)	ORR* (mutations vs wild type)	TTP* (months; mutations vs wild type)	Median survival (months; mutation vs wild type)
Lynch[40]	8/9 (89)	—	—	—
Paez[39]	5/5 (100)	—	—	—
Huang[44]	7/9 (78)	—	—	—
Cappuzzo[67]	8/12 (67)	53% vs 5%	9.9 vs 2.6 (p=0.02)	20.8 vs 8.4 (p=0.09)
Eberhard[47]	8/26 (31)	53% vs 18%	8 vs 5 (p<0.001)	Not reached vs 10 (p<0.001)
Mitsudomi[42]	24/26 (92)	83% vs 10%	—	Significantly longer (p=0.005)
Taron[50]	16/22 (73)	94% vs 13%	—	Not reached vs 9.9 (p<0.001)
Shih[49]	20/23 (87)	69% vs 9%	9 vs. 2.2 (p=0.001)	13.9 vs 4.8 (p=0.013)
Takano[70]	32/35 (91)	82% vs 11%	12.6 vs 1.7 (p<0.001)	20.4 vs 6.9 (p<0.001)

*ORR, objective response rate (complete response or partial response); TTP, time to progression.
—, data are not available.

SUMMARY AND FUTURE RESEARCH DIRECTIONS

The discovery of somatic *EGFR* mutations has been claimed as a big victory of molecular medicine.[41] It has had a considerable effect on the treatment of NSCLC. The strong associations between the mutations and gefitinib responsiveness and favourable prognosis provide forceful support for an individual genotype-based therapeutic strategy. By now, a number of genotyping methods have been developed. However, most of them are technically complicated and need substantial research resources and expertise, which make routine clinical screening difficult and impractical. Developing a set of simple and accurate genotyping methods would be of great importance for translating this bench discovery into clinical application. On the other hand, further basic studies are required to investigate whether other functional consequences are present downstream of EGFR signalling pathways and whether additional predictive biomarkers are available. For patients with acquired resistance to EGFR-TKIs, monitoring the *EGFR* mutation status in recurrent tumours is crucial for revealing the molecular mechanisms of drug resistance and developing new generations of TKIs. Finally, more large prospective studies to evaluate the therapeutic and prognostic value of *EGFR* mutations are required in patients with *EGFR*-mutation-enriched NSCLC. Prospective study is usually superior to retrospective study in bias and confounder control and therefore can provide more accurate assessment of the significance of *EGFR* mutations in the clinical treatment of NSCLC and of the possibility that EGFR-TKIs might replace conventional chemotherapy as the preferred antitumour drugs for the subset of patients with NSCLC.

Gealy R, Zhang L, Siegfried JM, *et al*. Comparison of mutations in the p53 and K-ras genes in lung carcinomas from smoking and nonsmoking women. *Cancer Epidemiol Biomarkers Prev* 1999;**8**(Pt 1):297–302.

Authors' affiliations

Xiaozhu Zhang, Division of Biomedical Sciences, Johns Hopkins Singapore, Singapore
Alex Chang, International Medical Centre, Johns Hopkins Singapore, Singapore

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REFERENCES

1 **Parkin DM**. Global cancer statistics in the year 2000. *Lancet Oncol* 2001;**2**:533–43.
2 **Greenlee RT**, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. *CA Cancer J Clin* 2001;**51**:15–36.
3 **Brambilla E**, Travis WD, Colby TV, Corrin B, Shimosato Y. The new World Health Organization classification of lung tumours. *Eur Respir J* 2001;**18**:1059–68.
4 **Travis WD**, Travis LB, Devesa SS. Lung cancer. *Cancer* 1995;**75**(Suppl):191–202S.
5 **Franceschi S**, Bidoli E. The epidemiology of lung cancer. *Ann Oncol* 1999;**10**(Suppl 5):3–6.
6 **Lam WK**. Lung cancer in Asian women—the environment and genes. *Respirology* 2005;**10**:408–17.
7 **Doll R**, Peto R. Cigarette smoking and bronchial carcinoma: dose and time relationships among regular smokers and lifelong non-smokers. *J Epidemiol Community Health* 1978;**32**:303–13.
8 **Akiba S**, Hirayama T. Cigarette smoking and cancer mortality risk in Japanese men and women—results from reanalysis of the six-prefecture cohort study data. *Environ Health Perspect* 1990;**87**:19–26.
9 **Freund KM**, Belanger AJ, D'Agostino RB, Kannel WB. The health risks of smoking. The Framingham Study: 34 years of follow-up. *Ann Epidemiol* 1993;**3**:417–24.
10 **Sekido Y**, Fong KM, Minna JD. Progress in understanding the molecular pathogenesis of human lung cancer. *Biochim Biophys Acta* 1998;**1378**:F21–59.
11 **Fong KM**, Sekido Y, Minna JD. Molecular pathogenesis of lung cancer. *J Thorac Cardiovasc Surg* 1999;**118**:1136–52.
12 **Breuer RH**, Postmus PE, Smit EF. Molecular pathology of non-small-cell lung cancer. *Respiration* 2005;**72**:313–30.
13 **Shimizu N**, Behzadian MA, Shimizu Y. Genetics of cell surface receptors for bioactive polypeptides: binding of epidermal growth factor is associated with the presence of human chromosome 7 in human-mouse cell hybrids. *Proc Natl Acad Sci USA* 1980;**77**:3600–4.
14 **Coussens L**, Yang-Feng TL, Liao YC, Chen E, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J, Francke U, Levinson A, Ullrich A. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science* 1985;**230**:1132–9.
15 **Kraus MH**, Issing W, Miki T, Popescu NC, Aaronson SA. Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. *Proc Natl Acad Sci USA* 1989;**86**:9193–7.
16 **Plowman GD**, Culouscou JM, Whitney GS, Green JM, Carlton GW, Foy L, Neubauer MG, Shoyab M. Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. *Proc Natl Acad Sci USA* 1993;**90**:1746–50.
17 **Wahl MI**, Carpenter G. Role of growth factors and their receptors in the control of normal cell proliferation and cancer. *Clin Physiol Biochem* 1987;**5**:130–9.
18 **Carpenter G**. Receptors for epidermal growth factor and other polypeptide mitogens. *Annu Rev Biochem* 1987;**56**:881–914.
19 **Lemoine NR**, Lobresco M, Leung H, Barton C, Hughes CM, Prigent SA, Gullick WJ, Kloppel G. The erbB-3 gene in human pancreatic cancer. *J Pathol* 1992;**168**:269–73.
20 **Salomon DS**, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 1995;**19**:183–232.
21 **Schlessinger J**. Cell signaling by receptor tyrosine kinases. *Cell* 2000;**103**:211–25.
22 **Yarden Y**, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001;**2**:127–37.
23 **Scagliotti GV**, Selvaggi G, Novello S, Hirsch FR. The biology of epidermal growth factor receptor in lung cancer. *Clin Cancer Res* 2004;**10**(12 Pt 2):4227–32S.
24 **Mendelsohn J**, Baselga J. The EGF receptor family as targets for cancer therapy. *Oncogene* 2000;**19**:6550–65.
25 **Ennis BW**, Lippman ME, Dickson RB. The EGF receptor system as a target for antitumor therapy. *Cancer Invest* 1991;**9**:553–62.
26 **Pavelic K**, Banjac Z, Pavelic J, Spaventi S. Evidence for a role of EGF receptor in the progression of human lung carcinoma. *Anticancer Res* 1993;**13**:1133–7.
27 **Ohsaki Y**, Tanno S, Fujita Y, Toyoshima E, Fujiuchi S, Nishigaki Y, Ishida S, Nagase A, Miyokawa N, Hirata S, Kikuchi K. Epidermal growth factor receptor expression correlates with poor prognosis in non-small cell lung cancer patients with p53 overexpression. *Oncol Rep* 2000;**7**:603–7.
28 **Volm M**, Rittgen W, Drings P. Prognostic value of ERBB-1, VEGF, cyclin A, FOS, JUN and MYC in patients with squamous cell lung carcinomas. *Br J Cancer* 1998;**77**:663–9.
29 **Nicholson RI**, Gee JM, Harper ME. EGFR and cancer prognosis. *Eur J Cancer* 2001;**37**(Suppl 4):9–15S.
30 **Garcia de Palazzo IE**, Adams GP, Sundareshan P, Wong AJ, Testa JR, Bigner DD, Weiner LM. Expression of mutated epidermal growth factor receptor by non-small cell lung carcinomas. *Cancer Res* 1993;**53**:3217–20.
31 **Pedersen MW**, Meltorn M, Damstrup L, Poulsen HS. The type III epidermal growth factor receptor mutation. Biological significance and potential target for anti-cancer therapy. *Ann Oncol* 2001;**12**:745–60.
32 **Chu CT**, Everiss KD, Wikstrand CJ, Batra SK, Kung HJ, Bigner DD. Receptor dimerization is not a factor in the signalling activity of a transforming variant epidermal growth factor receptor (EGFRvIII). *Biochem J* 1997;**324**(Pt 3):855–61.
33 **Yamazaki H**, Ohba Y, Tamaoki N, Shibuya M. A deletion mutation within the ligand binding domain is responsible for activation of epidermal growth factor receptor gene in human brain tumors. *Jpn J Cancer Res* 1990;**81**:773–9.
34 **Ji H**, Zhao X, Yuza Y, Shimamura T, Li D, Protopopov A, Jung BL, McNamara K, Xia H, Glatt KA, Thomas RK, Sasaki H, Horner JW, Eck M, Mitchell A, Sun Y, Al-Hashem R, Bronson RT, Rabindran SK, Discafani CM, Maher E, Shapiro GI, Meyerson M, Wong KK. Epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors. *Proc Natl Acad Sci USA* 2006;**103**:7817–22.
35 **Cohen MH**, Williams GA, Sridhara R, Chen G, Pazdur R. FDA drug approval summary: gefitinib (ZD1839) (Iressa) tablets. *Oncologist* 2003;**8**:303–6.
36 **Siegel-Lakhai WS**, Beijnen JH, Schellens JH. Current knowledge and future directions of the selective epidermal growth factor receptor inhibitors erlotinib (Tarceva) and gefitinib (Iressa). *Oncologist* 2005;**10**:579–89.
37 **Fukuoka M**, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereislova A, Dong RP, Baselga J. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. *J Clin Oncol* 2003;**21**:2237–46.
38 **Kris MG**, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, Schiller JH, Kelly K, Spiridonidis H, Sandler A, Albain KS, Cella D, Wolf MK, Averbuch SD, Ochs JJ, Kay AC. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;**290**:2149–58.
39 **Paez JG**, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;**304**:1497–500.
40 **Lynch TJ**, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;**350**:2129–39.
41 **Marx J**. Medicine. Why a new cancer drug works well, in some patients. *Science* 2004;**304**:658–9.
42 **Mitsudomi T**, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, Hatooka S, Shinoda M, Takahashi T, Yatabe Y. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J Clin Oncol* 2005;**23**:2513–20.

43 **Shigematsu H**, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba, II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD, Gazdar AF. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;**97**:339–46.
44 **Huang SF**, Liu HP, Li LH, Ku YC, Fu YN, Tsai HY, Chen YT, Lin YF, Chang WC, Kuo HP, Wu YC, Chen YR, Tsai SF. High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. *Clin Cancer Res* 2004;**10**:8195–203.
45 **Yokoyama T**, Kondo M, Goto Y, Fukui T, Yoshioka H, Yokoi K, Osada H, Imaizumi K, Hasegawa Y, Shimokata K, Sekido Y. EGFR point mutation in non-small cell lung cancer is occasionally accompanied by a second mutation or amplification. *Cancer Sci* 2006;**97**:753–9.
46 **Marchetti A**, Martella C, Felicioni L, Barassi F, Salvatore S, Chella A, Camplese PP, Iarussi T, Mucilli F, Mezzetti A, Cuccurullo F, Sacco R, Buttitta F. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 2005;**23**:857–65.
47 **Eberhard DA**, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, Ince WL, Janne PA, Januario T, Johnson DH, Klein P, Miller VA, Ostland MA, Ramies DA, Sebisanovic D, Stinson JA, Zhang YR, Seshagiri S, Hillan KJ. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;**23**:5900–9.
48 **Sonobe M**, Manabe T, Wada H, Tanaka F. Mutations in the epidermal growth factor receptor gene are linked to smoking-independent, lung adenocarcinoma. *Br J Cancer* 2005;**93**:355–63.
49 **Shih JY**, Gow CH, Yu CJ, Yang CH, Chang YL, Tsai MF, Hsu YC, Chen KY, Su WP, Yang PC. Epidermal growth factor receptor mutations in needle biopsy/aspiration samples predict response to gefitinib therapy and survival of patients with advanced nonsmall cell lung cancer. *Int J Cancer* 2006;**118**:963–9.
50 **Taron M**, Ichinose Y, Rosell R, Mok T, Massuti B, Zamora L, Mate JL, Manegold C, Ono M, Queralt C, Jahan T, Sanchez JJ, Sanchez-Ronco M, Hsue V, Jablons D, Sanchez JM, Moran T. Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. *Clin Cancer Res* 2005;**11**:5878–85.
51 **Pao W**, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kris M, Varmus H. EGF receptor gene mutations are common in lung cancers from ''never smokers'' and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 2004;**101**:13306–11.
52 **Pao W**, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005;**2**:e73.
53 **Kobayashi S**, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG, Halmos B. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;**352**:786–92.
54 **Bell DW**, Gore I, Okimoto RA, Godin-Heymann N, Sordella R, Mulloy R, Sharma SV, Brannigan BW, Mohapatra G, Settleman J, Haber DA. Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. *Nat Genet* 2005;**37**:1315–16.
55 **Kosaka T**, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. cit-tl>Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res* 2004;**64**:8919–23.
56 **Soh J**, Toyooka S, Aoe K, Asano H, Ichihara S, Katayama H, Hiraki A, Kiura K, Aoe M, Sano Y, Sugi K, Shimizu N, Date H. Usefulness of EGFR mutation screening in pleural fluid to predict the clinical outcome of gefitinib treated patients with lung cancer. *Int J Cancer* 2006;**119**:2353–8.
57 **Asano H**, Toyooka S, Tokumo M, Ichimura K, Aoe K, Ito S, Tsukuda K, Ouchida M, Aoe M, Katayama H, Hiraki A, Sugi K, Kiura K, Date H, Shimizu N. Detection of EGFR gene mutation in lung cancer by mutant-enriched polymerase chain reaction assay. *Clin Cancer Res* 2006;**12**:43–8.
58 **Fan X**, Furnari FB, Cavenee WK, Castresana JS. Non-isotopic silver-stained SSCP is more sensitive than automated direct sequencing for the detection of PTEN mutations in a mixture of DNA extracted from normal and tumor cells. *Int J Oncol* 2001;**18**:1023–6.
59 **Bosari S**, Marchetti A, Buttitta F, Graziani D, Borsani G, Loda M, Bevilacqua G, Coggi G. Detection of p53 mutations by single-strand conformation polymorphisms (SSCP) gel electrophoresis. A comparative study of radioactive and nonradioactive silver-stained SSCP analysis. *Diagn Mol Pathol* 1995;**4**:249–55.
60 **Pan Q**, Pao W, Ladanyi M. Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. *J Mol Diagn* 2005;**7**:396–403.
61 **Nagai Y**, Miyazawa H, Huqun, Tanaka T, Udagawa K, Kato M, Fukuyama S, Yokote A, Kobayashi K, Kanazawa M, Hagiwara K. Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. *Cancer Res* 2005;**65**:7276–82.
62 **Sugio K**, Uramoto H, Ono K, Oyama T, Hanagiri T, Sugaya M, Ichiki Y, So T, Nakata S, Morita M, Yasumoto K. Mutations within the tyrosine kinase domain of EGFR gene specifically occur in lung adenocarcinoma patients with a low exposure of tobacco smoking. *Br J Cancer* 2006;**94**:896–903.
63 **Tang X**, Shigematsu H, Bekele BN, Roth JA, Minna JD, Hong WK, Gazdar AF, Wistuba, II. EGFR tyrosine kinase domain mutations are detected in histologically normal respiratory epithelium in lung cancer patients. *Cancer Res* 2005;**65**:7568–72.
64 **Greulich H**, Chen TH, Feng W, Janne PA, Alvarez JV, Zappaterra M, Bulmer SE, Frank DA, Hahn WC, Sellers WR, Meyerson M. Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. *PLoS Med* 2005;**2**:e313.
65 **Sordella R**, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 2004;**305**:1163–7.
66 **Tracy S**, Mukohara T, Hansen M, Meyerson M, Johnson BE, Janne PA. Gefitinib induces apoptosis in the EGFRL858R non-small-cell lung cancer cell line H3255. *Cancer Res* 2004;**64**:7241–4.
67 **Cappuzzo F**, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, Haney J, Witta S, Danenberg K, Domenichini I, Ludovini V, Magrini E, Gregorc V, Doglioni C, Sidoni A, Tonato M, Franklin WA, Crino L, Bunn PA Jr, Varella-Garcia M. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005;**97**:643–55.
68 **Hirsch FR**, Varella-Garcia M, McCoy J, West H, Xavier AC, Gumerlock P, Bunn PA Jr, Franklin WA, Crowley J, Gandara DR. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. *J Clin Oncol* 2005;**23**:6838–45.
69 **Reinmuth N**, Brandt B, Kunze WP, Junker K, Thomas M, Achatzy R, Scheld HH, Semik M. Ploidy, expression of erbB1, erbB2, P53 and amplification of erbB1, erbB2 and erbB3 in non-small cell lung cancer. *Eur Respir J* 2000;**16**:991–6.
70 **Takano T**, Ohe Y, Sakamoto H, Tsuta K, Matsuno Y, Tateishi U, Yamamoto S, Nokihara H, Yamamoto N, Sekine I, Kunitoh H, Shibata T, Sakiyama T, Yoshida T, Tamura T. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005;**23**:6829–37.
71 **Amann J**, Kalyankrishna S, Massion PP, Ohm JE, Girard L, Shigematsu H, Peyton M, Juroske D, Huang Y, Stuart Salmon J, Kim YH, Pollack JR, Yanagisawa K, Gazdar A, Minna JD, Kurie JM, Carbone DP. Aberrant epidermal growth factor receptor signaling and enhanced sensitivity to EGFR inhibitors in lung cancer. *Cancer Res* 2005;**65**:226–35.
72 **Tokumo M**, Toyooka S, Kiura K, Shigematsu H, Tomii K, Aoe M, Ichimura K, Tsuda T, Yano M, Tsukuda K, Tabata M, Ueoka H, Tanimoto M, Date H, Gazdar AF, Shimizu N. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. *Clin Cancer Res* 2005;**11**:1167–73.
73 **Shigematsu H**, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. *Int J Cancer* 2006;**118**:257–62.
74 **Groves C**, Lamlum H, Crabtree M, Williamson J, Taylor C, Bass S, Cuthbert-Heavens D, Hodgson S, Phillips R, Tomlinson I. Mutation cluster region, association between germline and somatic mutations and genotype-phenotype correlation in upper gastrointestinal familial adenomatous polyposis. *Am J Pathol* 2002;**160**:2055–61.
75 **Phillips DH**, Hewer A, Martin CN, Garner RC, King MM. Correlation of DNA adduct levels in human lung with cigarette smoking. *Nature* 1988;**336**:790–2.
76 **Brownson RC**, Alavanja MC, Caporaso N, Simoes EJ, Chang JC. Epidemiology and prevention of lung cancer in nonsmokers. *Epidemiol Rev* 1998;**20**:218–36.
77 **Gartner LP**, Hiatt JL. *Color text book of histology*. Philadelphia: WB Saunders, 2001.
78 **Inoue A**, Suzuki T, Fukuhara T, Maemondo M, Kimura Y, Morikawa N, Watanabe H, Saijo Y, Nukiwa T. Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. *J Clin Oncol* 2006;**24**:3340–6.
79 **Asahina H**, Yamazaki K, Kinoshita I, Sukoh N, Harada M, Yokouchi H, Ishida T, Ogura S, Kojima T, Okamoto Y, Fujita Y, Dosaka-Akita H, Isobe H, Nishimura M. A phase II trial of gefitinib as first-line therapy for advanced non-small cell lung cancer with epidermal growth factor receptor mutations. *Br J Cancer* 2006;**95**:998–1004.
80 **Jackman DM**, Yeap BY, Sequist LV, Lindeman N, Holmes AJ, Joshi VA, Bell DW, Huberman MS, Halmos B, Rabin MS, Haber DA, Lynch TJ, Meyerson M, Johnson BE, Janne PA. Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. *Clin Cancer Res* 2006;**12**:3908–14.
81 **Choong NW**, Dietrich S, Seiwert TY, Tretiakova MS, Nallasura V, Davies GC, Lipkowitz S, Husain AN, Salgia R, Ma PC. Gefitinib response of erlotinib-refractory lung cancer involving meninges—role of EGFR mutation. *Nat Clin Pract Oncol* 2006;**3**:50–7; quiz 1 p following 57.
82 **Engelman JA**, Mukohara T, Zejnullahu K, Lifshits E, Borras AM, Gale CM, Naumov GN, Yeap BY, Jarrell E, Sun J, Tracy S, Zhao X, Heymach JV, Johnson BE, Cantley LC, Janne PA. Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. *J Clin Invest* 2006;**116**:2695–706.
83 **Antonescu CR**, Besmer P, Guo T, Arkun K, Hom G, Koryotowski B, Leversha MA, Jeffrey PD, Desantis D, Singer S, Brennan MF, Maki RG, DeMatteo RP. Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. *Clin Cancer Res* 2005;**11**:4182–90.
84 **Shah NP**, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, Sawyers CL. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2002;**2**:117–25.
85 **Keohavong P**, DeMichele MA, Melacrinos AC, Landreneau RJ, Weyant RJ, Siegfried JM. Detection of K-ras mutations in lung carcinomas: relationship to prognosis. *Clin Cancer Res* 1996;**2**:411–18.
86 **Siegfried JM**, Gillespie AT, Mera R, Casey TJ, Keohavong P, Testa JR, Hunt JD. Prognostic value of specific KRAS mutations in lung adenocarcinomas. *Cancer Epidemiol Biomarkers Prev* 1997;**6**:841–7.
87 **Toyooka S**, Tsuda T, Gazdar AF. The TP53 gene, tobacco exposure, and lung cancer. *Hum Mutat* 2003;**21**:229–39.